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Determining The Coupling Between Subunits In Kcsa Using Single Channel Fluorescence Spectroscopy
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KcsA channels have been extensively studied to understand K+ permeation through ion channels. It has been postulated that the opening of the conducting pore at the bundle crossing of the four TM2 helices has to occur before ion conduction. It remains unclear whether this process happens in one cooperative step, as suggested for voltage-gated channels, or if the subunits can act independently. Here, we present a study of the subunits' cooperativity using single channel fluorescence spectroscopy. We have shown previously (Blunck et al., 2006) that we can monitor the movement of the TM2. This is done by labeling them at the C-terminus of TM2 with an environment sensitive dye. We introduced purified and labeled KcsA channels into supported lipid bilayer and imaged their fluorescence time course. Fluctuations in the fluorescence intensity prior to photobleaching were interpreted as the movement of the four subunits. The associated open probability followed the KcsA pH dependence, and mean values similar to our results in planar lipid bilayer were observed. We observed occurrence of simultaneous gating of all four subunits, but also independent gating of single subunits. We analyzed the time traces with a hidden Markov model, considering photobleaching and coupling energy between subunits, in order to quantify the coupling energy. We found a coupling energy with a mean value of about 2.5 kT, which seems to be slightly higher at low pH. Current results showed occurrence of subconductance levels which we hypothesize to be correlated with the partial opening of the lower gate. Therefore, we are currently working on simultaneously detecting current and fluorescence of a single KcsA channel in a planar lipid bilayer.

118-Plat
Light-gated Ion Channels Based on Gramicidin A
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The natural pore-forming peptide gramicidin A has been used for more than twenty years as a platform for sensing. In recent years, the drive has been towards progressively more sensitive detection, approaching the ability to detect single molecules. In an effort to expand the utility of ion channels derived from gramicidin A for sensing applications, we created a neoteric photo-gated gramicidin derivative containing a spiropyran functional group. Upon irradiation with a specific wavelength of light, spiropyrans undergo significant structural rearrangements, including the formation of a quaternary amine, which gives rise to a large (15D) change in dipole moment. We have exploited this unique chemistry by functionalizing the C-terminus of gramicidin with a spiropyran to demonstrate that conductance through these pores can be modulated as a function of the applied wavelength of light. We have also explored the effect of pH on the ion conductance of these light-gated channels. We demonstrate the exquisite responsiveness sensitivity of conductance through these gramicidin pores to various external factors, which may open the door for a number of niche sensing applications for these types of semi-synthetic ion channels.

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Lipid Channels: Positive and Negative Cooperativity and Regulation by Proteins
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The sphingolipid, ceramide, self-assembles in phospholipid membranes, forming large channels capable of translocating proteins across said membranes. These channels are believed responsible for protein release from mitochondria, a key, decision-making step in apoptosis. Rather unexpectedly for lipids, ceramide channels reveal a high degree of order and display properties similar to those of protein channels. They contain amide linkages believed to be responsible for organizing individual lipids into columns, a kind of secondary structure. The columns self-assemble into a barrel-stave, essentially quaternary structure, that is so highly-organized that it shows both negative and positive cooperativity in the presence of chemical analogs. Depending on the location of the chemical change, the presence of the analog could favor channel formation or inhibit the formation. Thus the analogs must intercalate into the structure, altering its stability. Furthermore, ceramide channels can be destabilized or stabilized by proteins that regulate apoptosis and these act in a very specific manner. These proteins do not act as catalysts but rather as modulators or allosteric modifiers. They act to shift the stability constants that determine the equilibrium between channels, monomers, and non-channel aggregates. Bcl-xL destabilizes the channels by forming what appears to be a 1:1 complex with the ceramide channel. The stoichiometry of the interaction with oligomeric Bax, the protein that stabilizes the channel, appears to be larger than 1:1 but the variable oligomeric nature of the protein complicates the interpretation. The affinity between oligomeric Bax and the ceramide channel seems to increase with channel size indicating the possibility of a conformation-driven enlargement of the channel. (supported by NSF grant: MCB-0641208 and a Nano-Biotechnology DBED award)

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Ryanodine Receptors Alter Store-Operated Ca^{2+} Influx In Resting And Activated Human T Cells By Regulating Ca^{2+} Retention Within The Store
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We have reported previously that in Jurkat T cells, ryanodine receptors (RyR) are activated by store-operated Ca^{2+} entry (SOCE). However, the role of RyR in Ca^{2+} signaling in human T cells has not been elucidated. Resting T cells were isolated from the peripheral blood of healthy volunteers and activated *in vitro* with anti-CD3/CD28 antibodies. Using RT-PCR analysis we found that both resting and activated human T cells express RyR1 mRNA and that expression levels were higher in activated T cells compared to the resting. Probing the functional expression of ryanodine (Ry)-sensitive store with caffeine, a RyR agonist, revealed that caffeine induced elevation in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in activated but not in resting T cells, indicating that Ry-sensitive store is upregulated after T cell activation. The caffeine-induced Ca^{2+} release was abolished with blocking concentrations of Ry. We further found that in both resting and activated T cells RyR blockers Ry and dantrolene reduced the rates and peak amplitudes of $[Ca^{2+}]_i$ transients induced by SOCE, while increasing store content. These indicate that RyR control SOCE by regulating Ca^{2+} retention within the store. Importance of RyR in regulation of Ca^{2+}-dependent factions of activated T cells was underscored by the fact that inhibition of RyR significantly downregulated activation-induced T cell proliferation. We conclude that upregulation of RyR is essential for maintaining of enhanced $[Ca^{2+}]_i$ signaling in activated T cells and that RyR may serve as a potential target for immunosuppression. Supported by AHA Grant-in-Aid 0755086Y to A.F.F.

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The Sodium Channel Accessory Subunit Navb1 Associates With Brain Kv4.2 And Modulates The Functional Expression Of Kv4.2-encoded Channels
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Somatodendritic A-type (IA) voltage-gated K+ (Kv) channels are key regulators of neuronal excitability, functioning to modulate the back-propagation of action potentials and the frequency of repetitive firing. Although progress has been made in identifying components of Kv4-encoded IA channels, increasing evidence suggests that additional regulators are involved in modulating the functional expression of these channels. To identify novel components of native Kv4.2-encoded IA channel complexes, an anti-Kv4.2 antibody was used to immunoprecipitate Kv4.2 from mouse brain and co-purifying proteins were identified using a mass spectrometry (MS)-based proteomic approach. In addition to the Kv4 pore-forming alpha subunits (Kv4.2, Kv4.3, Kv4.1) and the previously described accessory subunits KChIP1-4 and DPP6/DPP10, eight tandem MS spectra identifying the Navb1 peptide YENEVLQ LEEDERFEGR were acquired from Kv4.2 immunoprecipitates from wild-type brain lysates, but were absent from brains of animals (Kv4.2-/-) lacking Kv4.2. Although Navb1 has previously been reported to regulate the functional expression of voltage-gated Na+ channels in heterologous expression systems, these MS-based results suggest the interesting possibility that Navb1 might also function in the regulation of Kv4.2-encoded IA channels. To explore this possibility, the ability of Navb1 to modulate the functional expression of Kv4.2 channels was investigated in HEK-293 cells. Biochemical experiments confirmed that Navb1 co-immunoprecipitates with Kv4.2, but not with KChIP2. Whole-cell voltage-clamp recordings revealed that co-expression of Navb1 significantly increases Kv4.2-encoded current densities without measurably affecting the time- and/or voltage-dependent properties of the currents. Western blot and cell surface biotinylation experiments revealed that Navb1 increases the total and the cell surface expression of Kv4.2 proteins. Taken together, these results suggest that Navb1 associates with Kv4.2, increases the stability of the Kv4.2 protein and the cell surface expression of functional Kv4.2-encoded channels.